# Identifying Morphological and Functional Changes in a *Caenorhabditis elegans* Neuronal Aging Model of Huntington's Disease

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## Objective

- **Evaluate the effects of aging on neuronal morphology and gentle touch response in a C. elegans model of Huntington's disease**
- **Establish that our RNAi method in our two genetic strains, Huntington's disease model ID1 and control ID245, is selective and specific in silencing targeted genes in neurons**

## Introduction

- Aging is a major risk factor of developing neurodegenerative diseases
- Common hallmarks of neuronal aging:
  - -neuronal aberrations
  - -decline in synaptic activity
  - -accumulation of protein aggregates
  - -decline in short-term associative memory
- Increased neuronal aberrations with age have been observed in *Caenorhabditis elegans* (Pan et al, 2011; Tank et al, 2011)
- I am focusing on neuronal morphology with age in a Huntington's model of *C. elegans*
- Observing changes in six mechanosensory neurons that detect gentle touch: AVM, ALML, ALMR, PVM, PLML and PLMR
- All experiments are conducted at 25C with strains polyQ128 (Huntington's) and polyQ19 (control).

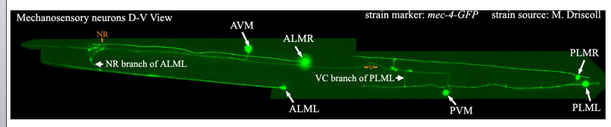



Figure 1: Locations of mechanosensory neurons AVM, ALML, ALMR, PVM, PLML and PLMR in *C. elegans*

## Hypothesis

**We hypothesize that an increase in neuronal aberrations and a decrease in touch responsiveness with age will be seen in both ID1 and ID245 strains, and that the ID1 strain will show a higher frequency of neuronal aberrations and less touch responsiveness than ID245.**

## Neuronal Assay

- An egg lay is conducted to obtain a synchronous population by transferring 20 worms at day one of adulthood onto a petri dish. After 4-5 hours all 20 worms are removed, leaving only eggs.
- Worms from egg lay are transferred beginning at day one of adulthood to keep the synchronous generation separate from progeny.
- Worms are imaged using a fluorescent microscope on day 3, 5, 7, 9, and 11 of adulthood.
- Aberrations are quantified and any significant difference in total aberration number between days in suspected to be due to aging


Figure 2: A) Healthy polyQ19 neuron at day 3 of adulthood. B) PolyQ19 neuron exhibiting multiple aberrations at day 7 of adulthood. C) PolyQ128 neuron exhibiting multiple aberrations at day 9 of adulthood.

## Touch Response

- Data is collected during each imaging time-point in the neuronal aging assay described above (days 3, 5, 7, 9, 11)
- An eyelash pick is used to stimulate worms at anterior and posterior locations
- The number of responses out of five are recorded from both locations for each worm
- Significant differences in percent touch responsiveness at specified time-points are expected to be due to aging.

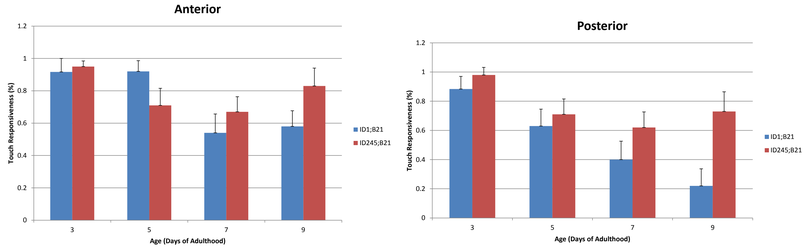



Figure 3: a) No significant difference in anterior touch responsiveness is seen between strains. b) Day 9 shows significant difference in posterior touch responsiveness between polyQ19 and polyQ128 (p value = 0.0143).

## RNAi Quality Control

- membrane via the Sid-1 transmembrane channel
- compared the fluorescents of two same-aged groups using the RNAi
- transferred C. elegans strain ID1;igls1[mec-3p::htt57-128Q::CFP;lin-15(t);mec-7p::YFP] onto two separate plates: the first containing L4440, and the other GFP RNAi.
- L4440 served as a vector control for RNAi
- GFP RNAi contains dsRNA coding for GFP
- Once absorbed the dsRNA would be converted into single strand RNAi and transported to the nucleus where it binds to the complementary strand
- Hypothesis: if our RNAi technique is effective, the experimental group will show a dimmer GFP signal than the control when the sample is imaged.
- Worms from both groups were imaged and quantified using imageJ to compare fluorescent intensity.

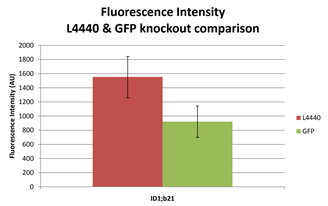
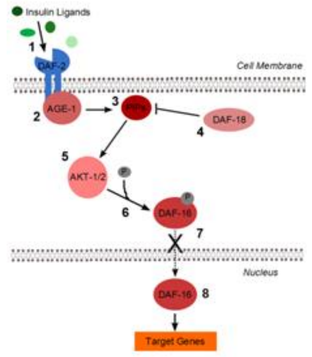



Figure 4: Significant difference in fluorescent intensity in polyQ128 strain (p value < .0001. GFP RNAi group is noticeably dimmer than control group L4440 RNAi.

## Future Directions

- Project goals:
  - -identify genes that affect neuronal aging, specifically those connected to the increase or decrease in neuronal aberrations with age and loss or gain of function in touch receptor neurons
  - -postulate their role in neuronal aging by comparing the results when each gene is expressed compared to when it is not
- Hypothesis:
  - -there are specific genes that correlate to the observable signs of neuronal aging that, when shut off, will have an effect on one or more hallmarks of aging
  - -expect that altering the expression of insulin ligands 1 through 39 will have an effect on neuronal aberrations which accumulate with age
- involve the use of RNAi to silence insulin signaling ligands, a group of hormones in the insulin family
- testing insulin receptor ligand genes (some of which are homologs of human insulin) 1 through 39
- insulin signaling pathway, which the insulin signaling ligands bind, has been shown to affect lifespan and protein aggregation in neurons in worms and higher organisms (Partridge et al, 2009)
- Observing the effects an absence of expression a gene has on neuronal morphology may allude to the relationship that gene has with the change in morphology over time

### Insulin-like signaling pathway in C. elegans




1. Extracellular insulin signaling ligands bind to the membrane bound insulin/IGF-1 receptor, DAF-2
2. Phosphatidylinositol 3-kinase AGE-1 is activated at cell membrane
3. AGE-1 then generates phospholipid signals, PIPs
4. Active DAF-18 phosphatase reduces the active amount of PIPs
5. PIPs activate the AKT family kinases, AKT-1 and AKT-2
6. Active AKT kinases phosphorylate the FOXO transcription factor DAF-16
7. DAF-16 is prevented from entering the nucleus
8. In the absence of insulin ligands and during heat shock and starvation, DAF-16 translocates into the nucleus and regulates the expression of genes for longevity, dauer, and stress resistance

## References

Pan et al. Genetic analysis of age-dependent defects of the Caenorhabditis elegans touch receptor neurons. PNAS 108(22):9274-9. (2011)

Partridge et al. Insulin/IGF-like signaling, the central nervous system and aging. (2009).

Tank et al. Spontaneous age-related neurite branching in Caenorhabditis elegans. Journal of Neuroscience 22;31(25):9279-88. (2011)

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